REMARKS AND ARGUMENTS

Status of the claims

Claims 19-23, 26 and 31-33 are currently pending and under examination in this application. Claims 19-23, 26 and 31-33 are all rejected in the office action of February 12th 2009.

Withdrawn objections and rejections

Applicant recognizes with satisfaction withdrawal of objection of the specification and rejection of claim 22 under 35 USC 112 second paragraph.

Claim rejection under 35 USC 112

Enablement

The Examiner rejects claims 23 and 26 under 35 USC 112 first paragraph, because the specification allegedly does not reasonably provide enablement for a therapeutic use of the fusion protein in any subject *in vivo* for any disease including cancer much less where the subject is human.

Applicant has amended claim 23, so as to narrow the claim to a recombinant fusion protein that is allowed to enter into human cell *in vitro* and inactivate of intracellular target of a scvFv-part of an antibody, where the fusion protein consists of the scFv-part of the antibody, a linker, and a cell penetrating transport peptide and the protein is a recombinant protein expressed in a host cell.

Applicant has cancelled claim 26.

Applicant submits that claim 23 as amended is enabled in the specification. For example in figure 3B it is shown that a recombinant fusion protein GST-GLI3(150-250)-9Arg internalizes into human 293 cells. Example 7 discloses that GL1-9Arg, -TP and -TP10 proteins entered into eukaryotic cells in culture and that the antibodies bound to the GL1 protein.

It has been shown in several publications that antibodies or antibody fragments bind and inhibit specific intracellular target proteins once in the cell. Intracellular antibodies (intrabodies) are a good example of such strategy (for review: Martin Stocks: Intrabodies as drug discovery tools and therapeutics. Curr. Op. In Chem. Biol. 92005) 9: 359-365, copy of the article attached). These antibodies are expressed within living target cells after cDNA encoding antibody fragment has been transfected. There is a major problem with *in vivo* application of intrabodies - it is impossible to transfect target cells systemically with cDNA encoding intrabodies. The instant invention teaches how to use antibodies *in vivo*.

Claim rejections under 35 USC section 102

Claims 19, 22, 23, and 26 are rejected under 35 USC Section 102 as being anticipated by Zhao et al. as evidenced by Pavlikova et al.

Applicant has amended the claims in previous office action reply so that the claims recite 'recombinant fusion protein' to overcome the rejection. The Examiner still maintains the rejection, because the skilled artisan would recognize that a scFv is itself a recombinant protein generated by recombinant technology from a parent antibody. Additionally the Examiner states that cell membrane penetrating peptides, transportan and transportan 10, are both art-recognized chimeric or recombinant fusion peptides. The Examiner points out that the claims do not distinguish which portion of the fusion protein is recombinant. The claims do not distinguish the recombinant scfv portion of the protein being conjugated or recombinant fused to a cell penetrating transport peptide. The claims do not distinguish a recombinant scFv antibody being chemically linked or recombinant fused with a recombinant cell penetrating peptide like transportan or tranportan 10. The Examiner concludes that accordingly Zhao as evidenced by Pavlikova teaches a recombinant fusion protein comprising a recombinant scFv antibody and a cell penetrating transport peptide associated with (conjugated to P) the scFv antibody, which anticipates the claims.

Applicant does not agree with the Examiner's view here. Claim 19 as amended previously recited 'a recombinant fusion protein' and applicant is of the opinion that it would be clear to one skilled in the art that the claimed protein in its entirety is a recombinant protein. However, to make the claim clear in this respect, **applicant has amended claims 19 and 20 so as to define that the recombinant fusion protein which consists of a scFv-part of an antibody, a linker and a cell penetrating transport peptide, is expressed from an expression vector cloned into a host cell.** This amendment should make it very clear that the full fusion protein is recombinant and not a product of conjugation of two portions as taught by Zhao. Accordingly, there is no anticipation by Zhao. Support for the claim amendment is found for example on application publication paragraph 0035.

Claims 19, 21-23, 26 and 33 are rejected under 35 USC 102 as being anticipated by Rothbard et al. In the previous office action reply, applicant argued that Rothbard et al teach Arg peptides that are conjugated with a fluorescent marker. The Examiner states that Rothbard teaching on page 13, lines 15-20 together with Rothbard's further disclosure of scFv and cell membrane transport peptides is reading at least in generic claim 19.

Applicant has amended claims 19 and 20, by clarifying that what is claimed is a recombinant fusion protein expressed from a vector cloned into a host cell. Rothbard does not teach recombinant fusion proteins and therefore the claims are not anticipated by Rothbard. Moreover, Applicant has replaced the verb 'comprising' with the verb 'consisting of', whereby a fluorescence marker cannot be a part of the protein here. As regards to claim 20, Rothbard does not teach GLI1 or GLI3.

Accordingly, Applicant request withdrawal of the rejection as the amended claims are not anticipated by Rothbard et al.

Regarding claim 21, the Examiner states that the Rothbard teaches subunit ranges for polyarginine peptides, which is considered to represent 'at least a part of Arg 9'. Amended claim 21 is dependent on the amended claim 20, which includes the limitation of GLI 1 of GLI3 and

recombinant fusion protein expressed from a vector cloned into a host cell, and therefore Rothbard is not anticipating the dependent claim.

Claim rejections under 35 USC 103

Claims 19, 20, 22, 23 and 26 are rejected under 35 USC 103(a) as being obvious over Zhao et al. as evidenced by Pavlikova et al,. in view of Toftgard.

The Examiner has considered applicant's previous arguments but does not find them persuasive. The Examiner states that both Zhao and Toftgard explicitly teach recombinant fusion protein comprising a recombinant scFv antibody where Toftgard further teaches linking these antibody fragments by recombinant technology to other protein molecules. Zhao teaches cellular uptake of a Mab conjugated with a cell penetrating peptide, and as evidenced by Pavlinkova, insertion of small molecules into a scFv antibody can be accomplished by cross-linking chemistry. The Examiner further states that a skilled artisan would recognize that a scFv is itself a recombinant protein generated by recombinant technology from a protein antibody. Thus the instant claimed 'recombinant fusion protein' comprises 'a recombinant scFv-part of an antibody'. The Examiner states that the claims do not distinguish which portion of the fusion protein is recombinant and that the claims do not distinguish the recombinant scFv portion of the protein being conjugated or recombinant fused to a cell penetrating transport peptide.

Applicant has amended claims 19, and 20 as discussed above to clarify that the fusion protein in expressed in host cell and is not a conjugate protein like the 5D10-MTS conjugate disclosed by Zhao.

Toftgard on the other hand teaches linking antibodies or antibody fragments by recombinant technology to other proteins; specifically Toftgard teaches peptides consisting of fragments of GLI-1 and SUFUH and monoclonal antibodies and antibody fragments specifically binding to these proteins.

Applicant contends that even if one skilled in the art would recognize that a scFv is itself a recombinant protein generated by recombinant technology from a parent antibody, she/he would not enter the instant invention as claimed in the amended claims by combining Toftgard teaching with the teaching with Zhao. Only by unpermitted hindsight can one conclude that one skilled in the art would have been motivated to have modified an antibody into a scfv and to express a peptide including the scfv, a linker and a cell membrane permeating peptide from a vector cloned into a host cell as is claimed in the amended claims. Accordingly, applicant respectfully request withdrawal of the rejection as it applies to claims 19, 20, 22, 23. Claim 26 is cancelled.

Obviousness rejection of claims 19-23, 26 and 33 is maintained over Zhao et al. as viewed by Pavlinkova et al. in view of Toftgard and in view of Rothbard and Lindgren et al. The Examiner also maintains the obviousness rejection of claims 19-23, 26, 31, and 33 over Rothbard in view of Lindgren. Moreover, the Examiner rejects claims 19-23, 26, 31 and 33 as being obvious over Rothbard in view of Lindgren and in further view of Toftgard.

All these rejections are discussed here together and the Declaration under 37 CFR 1.132 of the inventor , Prof. Kogerman, is incorporated by reference herein.

As is discussed above, Zhao et al. teaches conjugate proteins, Toftgard teaches peptides consisting of fragments of GLI-1 and SUFUH and monoclonal antibodies and antibody fragments specifically binding to these proteins. Rothbard teaches Arg9 as a transport peptide and Lindgren teaches transportan as transporter peptide.

The amended claims are toward a recombinant fusion protein that consists of scFv portion, a linker and a transport peptide expressed from a vector cloned into a host cell.

On page 19 of the outstanding office action the Examiner cites Rothbard from p. 13 lines 15-20. There Rothbard teaches that transport peptide polymers of his invention can be attached to biologically active polypeptide agents by recombinant means by constructing vectors for fusion proteins comprising the polypeptide or interest and the transport peptide.

Applicant points out here that the transport peptide polymers Rothbard teaches in the publication are poly-Arg peptides and the biologically active polypeptide agent encompasses almost any biologically active agents as is disclosed on page 4 lines 11-23 of Rothbard.

Applicant is of the opinion that such generic disclosure does not make the instant invention obvious when combined with the other prior art disclosures. Such generic disclosure would at most provide 'obvious to try' support for the rejection and that is not permissible. Applicant provided additional data in connection with the previous office action reply. The data is provided here as a declaration by the inventor. As is declared by Professor Priit Kogerman the data provided is used to show that the action of fusion proteins is not predictable; inventor shows that with different combinations and structures the action is different. As is stated by Prof. Kogerman in his declaration, the inventors have performed experiments with VL-TP-Linker-VH with linker peptides of various length. Applicant created recombinant fusion proteins that contained linkers as tandem repeats (2x linker and 3x linker sequence). The results of these experiments demonstrated that insertion of these longer sequences dramatically decreased the ability of fusion proteins to enter the cell. Most probably longer linkers hindered sterically transport peptide part of fusion protein. This is another demonstration that instant invention is not obvious.
Based on the above said the applicant maintains that creating a recombinant fusion protein that actually internalizes the cell and has the desired biological activity is not trivial. Therefore, the generic teaching of Rothbard in combination of the other prior art would not make the instant invention obvious.

Accordingly, applicant respectfully request withdrawal both of the rejection as they apply to claims 19-23, 31, and 33. Claim 26 has been cancelled.

Objection of the specification

The Examiner objects the legend of Figure 2, as having the same description for panels 2A and 2C and the same description for panels 2B and 2D. Applicant is requested to compare the

language between 2A and 2C, and d2C and 2D, which appears to be duplicative. Clarification is requested.

Applicant has amended the specification above to clarify this. Support to the amended specification is found in original application publication paragraph 0028.

New grounds for rejections

Claim 23 is rejected under 35 USC 101, because the claimed recitation of a use without setting forth any steps in the process, results in an improper definition of process. **Applicant has amended the claim so as to add steps where said protein is allowed to enter into human cells *in vitro* and inactivate an intracellular target of the scFv-part.** Support for this amendment is found in the original application for example in Example 7.

Claims 19-23, 26 and 31-33 are rejected under 35 USC 103(a) as being obvious over Rothbard in view of Toftgard and further in view of Lindgren et al and Soomets et al.

The Examiner states that the claimed recombinant fusion proteins were *prima facie* obvious at the time of the invention over Rothbard, Toftgard, Lingren and Soomets.

Regarding the claim interpretation set forth on p 24 of the outstanding office action, applicant has amended the independent claims 19, and 20 so that the 'comprising' language has been replaced by 'consisting of' language whereby the structure of the claimed proteins is not unlimited.

The Examiner rejects the claims as being prima facie obvious over the cited prior art. The Examiner states that Rothbard teaches poly –Arg peptides from 4-9 residues, or from 6-25 subunits for use as cell membrane transport peptides for selected agents across any number of biological membranes. Rothbard explicitly teaches delivering antibodies or antibody fragments such as scFv to the cytosol by attaching the transport polymers to the scFv, and that 'the

principle obstacle to wide application of this technology has been efficiency of uptake into infected cells'. Rothbard explicitly teaches fusion polypeptides comprising a polypeptide of interest and the transport peptide. Rothbard teaches that targets can be visualized with the fusion proteins and using the fusion proteins in pharmaceutical compositions.

The Examiner further states that Toftgard discloses the GLI-1 protein and the GLI-3 protein and making antibodies against these intracellular antigens. Included amongst the antibodies are single chain antibodies and pharmaceutical compositions comprising the antibodies and carriers. Toftgard teaches the technology for making protein fusion constructs in general.

Lindgren teaches cell penetrating peptides for transportan and the use of this and other peptides for cellular delivery of drugs or research tools.

Soomets teaches generating peptide mutants based on the wild-type transportan to generate transportan 10. Transportan 10 internalized to a comparable degree with transportan at different temperatures. The peptide was detected in the cytoplasm and nucleus of Bower cells, accumulating mainly in the intracellular membranous structures and nuclear envelope. Soomets teaches TP and TP190 penetrating into different cells in rapid and efficient way, the penetration is energy independent and not receptor mediated.

The Examiner now concludes that one skilled in the art would have been motivated and been reasonably assured of success in having produced a fusion protein comprising a scFv-cell membrane penetrating transport peptide at the time of the invention based on the combined disclosures of Rothbard, Toftgard, Lindgren and Soomets. Rothbard and Toftgard appreciate and expressly teach the utility of scFv antibody size in penetration tissues. Thus in order to visualize an intracellular antigen such as GLI-1 and GLI-3 as taught by Toftgard or an intracellular antigen of Rothbard, the ordinary artisan would have been motivated to have modified an antibody into a scFv not only to decrease the size, but to include a cell membrane permeating peptide such as taught by Rothbard and Lindgren and Soomets in order to facilitate cellular uptake of scFv antibody into a cell *in vitro*. In order to detect or visualize an intracellular antigen such as GLI1

or GLI3 as taught by Toftgard that was otherwise not accessible to an antibody without permealizing the cell itself, one would have been motivated to have engineered a fusion protein where Rothbard and Toftgard provided the methods for making fusion constructs for scFv and more especially Roghbard's teaching of scFv to include a cell penetrating peptide including arg9 and further in view of the peptides of Lingren and Soomets. The ordinary artisan would have been motivated at the time of the invention to engineer the cell penetration peptide to the scFv so that whole cells could be examined *in vitro* without affecting structure or viability. The ordinary artisan would have been motivated introducing the fusion protein of a diagnostic visualization of intracellular antigen expression in screening cell *in vitro* and where the fusion protein was formulated into a pharmaceutical composition compatible for administration to living cell *in vitro* to visualize GLI1 and GLI3. The ordinary artisan would have been reasonably assured of success in having produced or used the fusion protein for limited application *in vitro* because the methods and material for scfvs and cell penetrating peptides were already available based on the combined disclosures of the cited references, the construction of fusion proteins was already well known based on the combined disclosures of Rothbard and Toftgard, producing a scFv by introduction of a peptide in order to facilitate cellular uptake of the antibody had already been accomplished by Rothbard, and cell membrane penetration peptides were already known to be more effective than others based on Rothbard, Lindgren and Soomets. Based on this the Examiner concludes that the claimed fusion protein was *prima facie* obvious.

Applicant respectfully refutes this finding. Applicant is of the opinion that Examiner's conclusion of obviousness is based on improper hindsight reasoning and that the Examiner is applying an improper 'obvious to try' rational in support of an obviousness rejection.

Applicant incorporates here by reference the declaration of Professor Kogerman. As is shown in the declaration, it was not trivial (or obvious to that matter) to produce a recombinant fusion protein that includes the claimed elements and which internalizes the cell and has biological activity. Therefore, only with hindsight after reading the disclosure of the applicant's could one conclude that the invention as claimed in the amended claims was obvious. As is clear from the declaration of prof. Kogerman not each fusion protein would have the claimed activity. There is

no teaching whatsoever, in the prior art as to how a functional fusion protein should be constructed or which positions used. Therefore the rejection uses improper 'obvious to try' rational. The prior art does not give any indication of which of many choices would be successful. This is evidenced also by the enclosed declaration.

CONCLUSION

Applicant has addressed each of the rejections and objections made by the Examiner. Claims 19-21, 23, and 31-33 are amended and claim 26 is cancelled. Applicant believes that the amended application is in condition of allowance.

Applicant also invites the Examiner to call to the undersigned in case a telephone conference would be helpful to solve any remaining issues.

Respectfully,

DODDS AND ASSOCIATES

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